Primary structure and possible origin of the non-glycosylated basic proline-rich protein of human submandibular/sublingual saliva

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Human submandibular/sublingual saliva contains one non-glycosylated basic proline-rich protein whereas parotid saliva contains multiple such components. The submandibular protein has a primary structure identical with the C-terminal segment [TZ] of the human parotid acidic proline-rich proteins that contain 150 amino acid residues (M_r 16000). Northern-blot analyses of human parotid and submandibular glands revealed that mRNAs containing the *Hae*III repeat sequence typical for acidic proline-rich proteins are expressed in both of these salivary glands whereas mRNAs for non-glycosylated basic proline-rich proteins containing a typical *Bst*NI repeat sequence are expressed in the parotid but not in the submandibular gland. Products of translation *in vitro* of mRNAs from human parotid and submandibular glands were also examined. Two immunoprecipitable bands with M_r 29000 and 28000 were obtained by translation of both parotid and submandibular mRNA. In the presence of microsomal membranes these proteins gave rise to proteins electrophoretically identical with the secreted acidic proline-rich proteins of M_r 16000. These proteins were cleaved by kallikrein, giving rise to proteins with electrophoretic mobilities identical with those of a smaller acidic proline-rich protein with M_r 11000 and peptide TZ. Additional immunoprecipitable bands with M_r ranging from 35000 to 46000 were seen when parotid mRNA was used for translation *in vitro*, and are believed to be precursors of the basic proline-rich proteins encoded by the *Bst*NI repeat type mRNA. Neither these bands nor a separate precursor for the basic non-glycosylated proline-rich protein was detected when submandibular mRNA was used for translation *in vitro*. It is suggested that the non-glycosylated basic proline-rich protein present in human submandibular saliva arises by cleavage of acidic proline-rich proteins.

INTRODUCTION

Proline-rich proteins constitute over 70 % of the total protein in the human parotid-gland secretion [1]. They are categorized as acidic [2,3], non-glycosylated basic [1,4] and glycosylated basic [5,6], representing approx. 30 %, 23 % and 17 % respectively of the protein secreted by the human parotid gland [1]. They have been extensively studied with respect to their structures, functions and genetic relationships. The genes for proline-rich proteins (PRP) can be subdivided into an *Hae*III-type subfamily, which encodes acidic PRP (APRP), and a *Bst*NI-type subfamily, which codes for non-glycosylated basic PRP (BPRP) and glycosylated basic PRP [7]. References to these studies can be found in recent review articles by Bennick [8] and Minaguchi & Bennick [9]. Human submandibular/sublingual (HSMSL)-gland secretions have been shown to contain acidic proline-rich proteins [10], but these have not yet been fully characterized. No reports have been published to date concerning basic proline-rich proteins in human submandibular secretions. We report in the present paper the purification and characterization, including the amino

acid sequence, of the major BPRP found in HSMSL saliva. We also report the results of Northern-blot analysis and translation experiments *in vitro* showing that mRNAs of the *Hae*III type are expressed in both human parotid and submandibular glands whereas mRNAs of the *Bst*NI type are expressed only in parotid glands. These findings indicate that the submandibular BPRP may be a post-ribosomal cleavage product of APRP.

MATERIALS AND METHODS

Materials

Rabbit reticulocyte lysate (micrococcal-nuclease-treated) was purchased from Bethesda Research Laboratory (Gaithersburg, MD, U.S.A.), guanidinium thiocyanate was from Fluka Chemical Corp. (Ronkonkoma, NY, U.S.A.) and rabbit anti-(goat IgG) antibodies were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Calf-liver tRNA and oligo(dT)-cellulose were purchased from Boehringer Mannheim (Dorval, P.Q., Canada). [³H]Proline and [³⁵S]methionine of the highest specific

Abbreviations used: HSMSL, human submandibular/sublingual; PRP, proline-rich protein; BPRP, non-glycosylated basic proline-rich proteins; APRP, acidic proline-rich proteins; PIF, parotid isoelectric-focusing variant of APRP; 1 × SSC, 0.15 M-NaCl/0.015 M-sodium citrate buffer, pH 7.0; nt, nucleotide residue(s).

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These sequence data have been submitted to the EMBL/GenBank Data Libraries.

radioactivity available, sodium cyanoboro[^3H]hydride and dog pancreatic microsomal membranes were obtained from Amersham (Arlington Heights, IL, U.S.A.). Mixtures of acidic PRPs, PRP1 and PIFs (previously referred to as protein C), as well as of PRP3 and PIFf (also called protein A), were purified as described previously [10]. The 44-residue C-terminal TZ peptide of PRP1 was purified as described previously [11]. Kallikrein was obtained by the method of Wong *et al.* [12]. Radiolabelled APRPs and peptide TZ markers were prepared by reductive methylation [13]. Immunoglobulin fractions of normal goat serum as well as goat serum containing antibodies to APRP was purified by DEAE-cellulose column or affinity chromatography [14]. The probe containing *Hae*III repeats encoding basic PRPs, 2GI [15], and the other containing *Bst*NI repeats encoding basic PRPs, PRP1 2.2 RP [16], were kindly provided by Dr. N. Maeda (University of Wisconsin, Madison, WI, U.S.A.).

Collection of saliva

HSMSL saliva was collected from six subjects by using the Truelove device [17], the flow being stimulated with citric acid-saturated paper discs on the tongue. Samples (1 ml) were collected and immediately chilled. The proteinase inhibitor phenylmethanesulphonyl fluoride (final conc. 2 mM) and EDTA (final conc. 5 mM) were added immediately after collection. Parotid-gland saliva was collected from the same six subjects by using the double-chambered Curby cup, with sour lemon drops as the stimulant.

Purification of the major BPRP in HSMSL saliva

Approx. 100 ml of HSMSL saliva from each of two individuals was collected over a period of several weeks. Collections were treated as described above and stored at -20°C until use. The saliva from each individual was purified separately. The procedures used were the same as those employed for the purification of the parotid BPRP [1]. Briefly, this consisted of fractionation with 45%-saturated $(\text{NH}_4)_2\text{SO}_4$, gel filtration and ion-exchange chromatography on DEAE-Sephadex and SP-Sephadex.

Gel electrophoresis

Cationic slab-gel electrophoresis was performed by the method of Jordan & Raymond [18] as modified by Anderson *et al.* [19].

Amino acid analysis

Proteins were hydrolysed and analysed by AAA Laboratories, Mercer Island, WA, U.S.A. Hydrolysis was performed in 6 M-HCl at 110°C for 24 h.

N-Terminal analysis

The N-terminal amino acid was determined by using the dansyl procedure for peptides [20].

C-Terminal analysis

The C-terminal was determined by using carboxypeptidase Y (Pierce Chemical Co., Rockford, IL, U.S.A.). Digestion was done in 0.1 M-pyridine/acetate buffer, pH 6, at 37°C for 6 h. The weight ratio of enzyme to substrate was 1:20. The enzyme was inactivated by heating at 100°C for 5 min, and the digest was subjected to analysis on the amino acid analyser.

Clostripain digestion

The digestion was carried out in 0.05 M-ammonium bicarbonate buffer, pH 8, containing 25 mM-dithiothreitol at 37°C for 3 h. Peptides were purified by paper electrophoresis at pH 1.9 (4 kV for 25 min), and were eluted from the paper with 0.05 M-ammonium bicarbonate buffer, pH 8.

Sequence analysis

Automated sequencing was performed on the gas-phase protein Sequenator (model 470) from Applied Biosystems. Typically, 2 nmol samples were sequenced at least twice. The standard program provided by the company was used for sequence analysis.

Isolation of mRNA

A total of five parotid glands and six submandibular glands were obtained from 11 patients undergoing parotidectomy or laryngectomy and neck dissection at the Toronto General Hospital. The glands were stored in liquid N_2 immediately after removal.

Total RNA was isolated from the glands by the method of Harding *et al.* [21] except that the RNA was purified by repeated resuspension and subsequent precipitation with sodium acetate and ethanol [22]. The polyadenylated RNA was prepared from total RNA by oligo(dT)-cellulose chromatography [23,24].

RNA gel electrophoresis

mRNA was denatured, separated by electrophoresis in 1% agarose gel and transferred on to Biotrans nylon membrane (0.2 μm mesh) (ICN, Irvine, CA, U.S.A.) as described by Thomas [25] except that the mRNA was cross-linked by u.v. light by placing the wet membrane with transferred RNA 20 cm away from a germicidal lamp and exposing it to u.v. light for 10 min before baking of the membrane at 80°C for 2 h.

Hybridization method for Northern-blot analysis

The probes for this experiment were prepared from eluted insert DNA (approx. 0.7 kb from plasmid 2GI containing *Hae*III repeats encoding for acidic PRP and 2.2 kb from plasmid PRP1 2.2 RP containing *Bst*NI repeat encoding for basic PRP) using the random primer method [26].

The nylon membrane was prehybridized at 42°C with diethyl pyrocarbonate-treated Blotto (0.25%) in 50% (v/v) formamide in $6\times\text{SSC}/1\%$ (w/v) SDS containing 250 μg of single-stranded sheared salmon testis sperm DNA/ml for 18 h [27]. The membrane was then hybridized for 24 h with freshly prepared prehybridization solution, containing the probe, at 68°C . After hybridization the membrane was washed directly at a high-stringency condition with $2\times\text{SSC}/0.5\%$ SDS at room temperature for 5 min and with $2\times\text{SSC}/0.1\%$ SDS at room temperature for 15 min followed by washing with $0.1\times\text{SSC}/0.02\%$ SDS at 68°C for 60 min. It was then exposed to an X-ray film.

Translation of human parotid or submandibular mRNA *in vitro*

The polyadenylated RNA was translated *in vitro* by rabbit reticulocyte lysate [28]. To determine the extent of protein synthesis 2 μl samples were removed and 20%-(w/v)-trichloroacetic acid-insoluble radioactivity was

measured on filter-paper discs. The same method was used to monitor the [^3H]proline incorporation except that glass-fibre filter discs were used and the proteins were precipitated with 25% (w/v) trichloroacetic acid containing 2.5% (w/v) phosphotungstic acid.

Translation *in vitro* was also carried out in the presence of dog pancreatic microsomal membranes (5 μl , 1:10 dilution of vesicles as supplied) in a final reaction volume of 50 μl containing 20 μg of mRNA. After incubation the translation mixture was made 2% (w/v) with respect to SDS [29], heated at 100 °C for 2 min to disrupt the vesicles and centrifuged at 164000 g for 60 min at 3 °C. The radiolabelled proteins were precipitated with acetone. In some experiments the acetone-insoluble polypeptides were redissolved in 200 μl of 1% (w/v) NH_4HCO_3 , then precipitated with antibodies to acidic PRP, and the precipitate was dissolved in 1% NH_4HCO_3 and the redissolved immunoreactive protein was digested with 20 μl of kallikrein (0.2 ng/ml) in the presence of 0.02% NaN_3 at 22 °C for 96 h.

Immunoprecipitation of proteins translated *in vitro*

The translation mixture was centrifuged at 164000 g for 60 min at 3 °C. Affinity-purified antibodies to APRP were coupled to Affigel-10 beads according to the manufacturer's instructions. The beads were suspended in 10 mM-Tris/HCl buffer, pH 7.5, containing 150 mM-NaCl, 0.5% Nonidet P-40 and 0.02% NaN_3 (NTS buffer), and a 50 μl volume of packed beads was added to 20 μl of the translation mixture. After incubation at 4 °C for 3 h the mixture was centrifuged at 12000 g for 45 s. The beads were washed first in NTS buffer containing 1 mg of bovine serum albumin/ml and then in NTS buffer. The antigen was eluted from the beads by heating for 5 min in 10 mM-Tris/HCl buffer, pH 7.0, containing 1% (w/v) SDS. In some experiments a 12 μl portion of the supernatant translation mixture was added to a solution of antibodies to APRP (2 $\mu\text{g}/10 \mu\text{l}$) and the mixture was incubated for 2 h at 37 °C and left for 16 h at 4 °C. To facilitate the precipitation of immune complexes, a solution of rabbit anti-(goat IgG) antibodies was added and the incubation was continued at 4 °C for 16 h. After centrifugation, the pellet was washed four times with the buffer and dissolved by heating at 100 °C for 5 min in 30 μl of 50 mM-Tris/HCl buffer, pH 6.8, containing 6 M urea, 2% (w/v) SDS and 5% (v/v) 2-mercaptoethanol. The proteins produced by the translation *in vitro* and immune complexes were analysed by SDS/polyacrylamide-gel electrophoresis according to the procedure of Laemmli [30]. Slab gels (1.5 mm $\times$ 13 cm $\times$ 15 cm) were electrophoresed at 20 V until the Bromophenol Blue dye was 1 cm from the bottom of the gel. The gels were prepared for fluorography [31] and radioactive proteins were detected by exposing the dried gels to Kodak X-omat AR film at -80 °C for several days before they were developed.

RESULTS AND DISCUSSION

Fig. 1 shows the cationic gel-electrophoresis patterns of parotid saliva (Fig. 1a) and HSMSL saliva (Fig. 1b) from six individuals. All parotid-saliva samples exhibit multiple BPRP bands, as previously reported [1]. In contrast, only one major protein band can be seen in the area of the BPRP in the HSMSL samples. This band has

Fig. 1. Cationic gel electrophoresis of (a) human parotid saliva and (b) HSMSL saliva

(a) Parotid saliva from six individual subjects. The bracket indicates the area containing the BPRP. (b) HSMSL saliva from the same six subjects. The arrow indicates the position of the parotid protein IB-8b.

a mobility similar to the protein in parotid saliva designated IB-8b [1].

The chromatographic profiles of the purification steps used to purify the major BPRP are shown in Fig. 2. The dialysed 45%-satn.-(NH_4) $_2\text{SO}_4$ supernatant fraction from 100 ml of HSMSL saliva was applied to DEAE-Sephadex (Fig. 2a) and the breakthrough fractions were pooled as indicated (peak I). After freeze-drying, peak I was applied to a Bio-Gel P-10 column (Fig. 2b). Two fractions were eluted; peak I-A contains a basic proline-rich glycoprotein, as confirmed by amino acid and carbohydrate analyses, and peak I-B contains the non-glycosylated BPRP. These two basic proline-rich fractions constitute approx. 14% of the total proline-rich components in HSMSL saliva, whereas in parotid saliva they constitute 59% of the total. Peak I-B was further purified by SP-Sephadex chromatography (Fig. 2c). In contrast with the multiple BPRPs observed in SP-Sephadex chromatograms of human parotid saliva [1], HSMSL saliva contains only one major BPRP.

Compositional studies, presented in Table 1, show that HSMSL BPRP is a 44-amino acid-residue polypeptide containing no carbohydrate and with glycine as the *N*-terminal and glutamine as the *C*-terminal. The amino acid sequence of this BPRP is shown in Fig. 3. The sequence is identical with the *C*-terminal segment named TZ (residues 107–150) of human parotid acidic PRP1 and PRP2 and PIFs [11,32,33]. An identical 44-amino acid-residue BPRP has been isolated from human parotid saliva by Isemura *et al.* [34] and by us (D. L. Kauffman, M. Blum, A. Bennick & P. J. Keller, unpublished work).

The finding that the primary structure of the HSMSL BPRP corresponds to that of the *C*-terminal segment of APRP present in human parotid saliva raised the question of the nature of the mRNA encoding the HSMSL BPRP. Northern-blot analysis was therefore undertaken with mRNA from human parotid and submandibular glands and probes for acidic PRP containing *Hae*III repeats and basic PRP containing *Bst*NI repeats. This experiment is presented in Fig. 4.

With the *Bst*NI probe for basic PRP no hybridization was seen with submandibular mRNA, but parotid mRNA contained hybridizing bands varying from 800 nt

Fig. 2. Chromatographic profiles of the steps used to purify the major BPRP

In each panel the bar indicates fractions combined. (a) DEAE-Sephadex A-25 chromatography: column size, 2.5 cm $\times$ 90 cm; buffer, 0.05 M-Tris/HCl, pH 8.5 at 5 °C; flow rate, 12 ml/h; fraction volume, 4 ml. The arrow signifies application of 1 M-NaCl on to the column. (b) Bio-Gel P-10 gel filtration of DEAE-Sephadex peak I: column size 2 cm $\times$ 80 cm; buffer, 0.05 M-ammonium bicarbonate, pH 8; flow rate, 12 ml/h; fraction volume, 6 ml. (c) SP-Sephadex C-25 chromatography of Bio-Gel P-10 peak B: column size, 1.6 cm $\times$ 85 cm; gradient of 300 ml of 0.05 M-NaCl/0.015 M-sodium acetate buffer, pH 3.6, and 300 ml of 0.3 M-NaCl/0.015 M-sodium acetate buffer, pH 3.6, started after sample application; flow rate, 12 ml/h; fraction volume, 4 ml.

to 1680 nt, and their size varied in the two preparations, indicating polymorphism. With the *Hae*III probe for acidic PRP, prominent 800 nt bands were detected in all preparations of parotid and submandibular mRNA preparations. Two additional minor bands of 600 nt and 520 nt were also seen with the parotid mRNA preparations. These are probably degradation products,

Table 1. Amino acid composition of the HSMSL BPRP

The amino acid composition is given as residues per molecule by amino acid analysis or (in parentheses) from the sequence (Fig. 3). The serine value has been corrected by 10 % for hydrolysis loss. The *N*-terminal is glycine and the *C*-terminal is glutamine.

Amino acid	Amino acid composition (residues/molecule)	
Ser	1.2	(1)
Glx	10.1	(11)
Pro	17.7	(17)
Gly	10.8	(11)
Lys	1.0	(1)
His	0.9	(1)
Arg	1.9	(2)

Fig. 3. Summary proof and amino acid sequence of the major BPRP of HSMSL saliva

→, automated Edman degradation; ←, carboxypeptidase Y digestion. Sites of cleavage with clostripain are indicated by vertical arrows.

Fig. 4. Northern-blot analysis of parotid and submandibular mRNAs under high-stringency conditions

Probes were prepared from eluted insert DNA. Washing conditions were: 2 $\times$ SSC/0.5 % SDS at 22 °C for 5 min, then 2 $\times$ SSC/0.1 % SDS at 22 °C for 15 min, followed by 0.1 $\times$ SSC/0.02 % SDS at 68 °C for 60 min. (a) Eluted insert, *Hae*III repeat clone; (b) eluted insert, *Bst*NI repeat clone. P1 and P2, parotid mRNA; S1 and S2, submandibular mRNA. Markers: wheat-germ 18 S and 26 S RNA.

on the basis of their weak intensity relative to that of the prominent hybridizing band of 800 nt. Faint hybridization also was seen with parotid mRNA corresponding to bands observed in hybridization with the probe for basic-PRP mRNA, suggesting that under these conditions there is a weak non-specific hybridization.

mRNAs from human parotid and submandibular glands were examined further by means of translation *in vitro*. Experiments performed with [35 S]methionine (Fig. 5) showed that incorporation was enhanced approx. 8-fold by the presence of mRNA. SDS/polyacrylamide-gel-electrophoretic analyses of acetone-precipitable polypeptides revealed multiple labelled bands ranging in apparent M_r from 14300 to 87000. The translation products of both parotid and submandibular mRNAs included two components with M_r 28000 and 29000 that were immunoprecipitated with antibodies to human parotid APRP. Additional immunoprecipitable bands with M_r ranging from 35000 to 46000 were seen in the translation products of parotid mRNA, but not with submandibular mRNA. Antibody to human APRP is

known to cross-react with basic PRP, and, to date, it has not been possible to obtain antibodies specific for the acidic or basic PRP. However, in view of the results of the Northern blots, it is likely that the larger proteins (M_r 35000–46000) are translation products of the *Bst*NI-type mRNA coding for BPRP and that the 28000- M_r and 29000- M_r proteins are translation products of the *Hae*III-type RNA coding for APRP.

Translation *in vitro* was repeated with [3 H]proline and the results are shown in Fig. 6. With parotid mRNA extensive incorporation of 3 H was seen in several proteins ranging in M_r from 28000 to 58000. In contrast, with submandibular mRNA, no heavily labelled bands larger than M_r 29000 were observed. Addition of anti-APRP serum to proteins produced by translation of submandibular mRNA resulted in the precipitation of two proteins with M_r 29000 and 28000.

When translation was done in the presence of microsomal membranes, the intensities of the 29000- M_r and 28000- M_r bands were decreased and two new immunoprecipitable bands appeared, with electro-

Fig. 5. Analysis by electrophoresis on a 12.5% polyacrylamide gel and fluorography of polypeptides from translations *in vitro* with [35 S]methionine and polyadenylated RNA from parotid and submandibular glands

Lanes 1 and 4, labelled polypeptides obtained by translation of polyadenylated RNA from parotid glands from two individuals. Lanes 2 and 5, polypeptides isolated by immunoprecipitation with goat anti-APRP serum from the translation depicted in lanes 1 and 4 respectively. Lane 3, translation product precipitated with normal goat serum. Lane 6, M_r markers (M_r in descending order 97400, 68000, 43000, 25700, 18400 and 14300). Lane 7, mixture of PRP1 and PIFs. Lanes 8 and 10, polypeptides obtained by translation of submandibular mRNA. Lanes 9 and 11, polypeptides isolated by immunoprecipitation of the translation products shown in lanes 8 and 10 respectively. Affigel-linked antibodies were used in the experiments shown in lanes 2, 6 and 9, and rabbit anti-(goat IgG) antibodies were used in lane 11. The shadow in the lower right corner of lane 9 is due to overlapping material in the adjacent lane, which was removed.

Fig. 6. Analysis by electrophoresis on 15% polyacrylamide gels and fluorography of polypeptides from translations *in vitro* with [3 H]proline and polyadenylated RNA from parotid and submandibular glands

Lane 1, labelled polypeptides obtained by translation of polyadenylated RNA from a parotid gland. Lane 2, M_r markers (same M_r values as in Fig. 5). Lane 3, polypeptides obtained by translation of submandibular mRNA. Lane 4, polypeptides isolated by immunoprecipitation with normal goat serum. Lane 5, polypeptides isolated by immunoprecipitation of the translation products shown in lane 3 with anti-APRP antibodies coupled to Affigel. Lane 6, mixture of PRP1 and PIFs. Lane 7, mixture of PRP3 and PIFf. Lane 8, polypeptides obtained by translation of submandibular mRNA in the presence of microsomal membranes.

phoretic mobilities similar to those of secretory APRP1 and PIFs (M_r 16000). No protein bands corresponding to the putative post-translational derivatives APRP3 and PIFf (M_r 11000) and peptide TZ were seen, demonstrating the absence of separate mRNAs for these proteins. The apparent M_r values for the APRPs as measured from the gel are approx. 8000–9000 greater than the true M_r values, but it is well established that PRPs have abnormally low mobility on SDS/polyacrylamide gels.

Proteins formed by translation *in vitro* of submandibular mRNA in the presence of microsomal membranes and subsequently digested with kallikrein gave rise to an additional band with same electrophoretic mobility as PRP3 and PIFf (Fig. 7). It was not possible to discern the presence of a peptide with the same rate of migration as the C-terminal peptide TZ, owing to the presence of contaminating material. Immunoprecipitation of the translation product digested with kallikrein showed the presence of bands with mobilities of PRP3 and TZ in addition to the heavily labelled band corresponding to PRP1. The immunoprecipitation bands corresponding to APRP3 and peptide TZ were not observed when the translation product was incubated without kallikrein.

Several lines of evidence suggest that the non-glycosylated BPRP present in HSMSL saliva is not synthesized as such in the submandibular gland, but, rather, derives from an acidic PRP. The first of these is

Fig. 7 SDS/polyacrylamide-gel electrophoresis (15% gel) and fluorography of polypeptides obtained by translation *in vitro* of submandibular polyadenylated RNA in the presence of dog pancreatic microsomal membranes followed by digestion with salivary kallikrein

Lane 1, polypeptides synthesized *in vitro* in the presence of dog pancreatic microsomal membranes. Lane 2, mixture of PRP1 and PIFs. Lane 3, polypeptide shown in lane 1 after kallikrein digestion. Lane 4, peptide TZ. Lane 5, polypeptides isolated by immunoprecipitation of the products shown in lane 3. Lane 6, mixture of PRP3 and PIFf.

the fact that mRNA for basic PRPs containing *Bst*NI repeats is not demonstrable with Northern-blot analysis in mRNA preparations from human submandibular glands, although mRNA containing *Hae*III repeats for acidic PRP is present. The second is the observation from the translation experiments *in vitro* that there are no separate mRNAs in submandibular glands for the 11000- M_r PRPs and the 44-residue BPRP described in the present paper and that the 16000- M_r protein can be cleaved by kallikrein to give products that are identical in electrophoretic mobility with the secretory 11000- M_r PRPs and peptide TZ. Moreover, APRPs have been demonstrated in gel-electrophoretic patterns of HSMSL saliva, and Bennick has reported that two of these, protein C (a mixture of PRP1 and PIFs) and protein A (a mixture of PRP3 and PIFf) correspond electrophoretically and immunochemically to their parotid counterparts [10]. It appears that there are only two genes for APRP [35], both of which are expressed in parotid glands, and it would therefore be expected that the APRPs in submandibular saliva are encoded by the same genes. Thus present evidence indicates that APRPs synthesized in parotid and submandibular glands in a given individual are identical, although APRPs from submandibular saliva have not yet been sequenced. It is also known that PRP1 and PRP2 can be converted into PRP3 and PRP4 by kallikrein-like enzymes and that the conversion is accompanied by elimination from PRP1 and PRP2 of a 44-amino acid-residue segment identical with that reported in the present paper [12]. Another explanation for the presence of the BPRP in HSMSL saliva would be that there is an additional *Hae*III-type mRNA in the submandibular gland that encodes a larger basic precursor for BPRP. Although this possibility cannot be eliminated at present, it may be noted that no clone coding for such a protein has been isolated from a parotid cDNA library [15].

It was surprising that the probe for BPRP (*Bst*NI) used in the present study did not hybridize with the mRNA from human submandibular gland, since a glycosylated BPRP was isolated from HSMSL saliva (Fig. 2b, peak A), and Maeda *et al.* [15] have shown that the parotid glycosylated BPRP also contains the *Bst*NI repeat regions. The structure of the glycosylated BPRP in HSMSL saliva is not known, and it may be sufficiently different from the major BPR glycoprotein in parotid saliva that the probes in this study do not hybridize with its mRNA. Alternatively, since the HSMSL saliva used in this study is a mixture of secretions from the submandibular and the sublingual glands, it is possible that the glycosylated BPRP is not synthesized in the submandibular gland but is a product of the sublingual gland. Whatever the explanation, it is clear that there is a differential expression of the genes for PRPs in the salivary glands, and it should be interesting to study the structural basis for these differences.

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