Biosynthesis of Salivary Proteins in the Parotid Gland of the Subhuman Primate, *Macaca fascicularis*

CELL-FREE TRANSLATION OF THE mRNA FOR A PROLINE-RICH GLYCOPEPTIDE AND PARTIAL AMINO ACID SEQUENCE AND PROCESSING OF ITS SIGNAL PEPTIDE

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Heather S. Belford‡‡, Elisa G. Triffleman‡, Gwyneth F. Offner‡, Robert F. Troxler‡‡, and Frank G. Oppenheim‡‡

From the ‡Department of Biochemistry, Boston University School of Medicine, and ‡Department of Oral Biology, Goldman School of Graduate Dentistry, Boston, Massachusetts 02118

The major anionic proline-rich proteins in the parotid and submandibular secretions of subhuman primates and man perform the important biological function of inhibiting crystal growth of calcium phosphate salts from saliva, which is supersaturated with calcium phosphate salts, thereby preventing excess deposition of hydroxyapatite on tooth surfaces. The present work was initiated as a first step towards investigating proline-rich protein biosynthesis in parotid glands using the subhuman primate, *Macaca fascicularis*, as a model system. RNA was isolated from macaque parotid glands and separated into poly(A)-enriched and poly(A)-deficient fractions by chromatography on oligo(dT)-cellulose. The mRNAs in both fractions promoted incorporation of radiolabeled amino acids into polypeptides in an mRNA-dependent reticulocyte lysate translation system. Five major proline-rich polypeptides were detected and one of these was shown to be the *in vitro* precursor of the major anionic macaque proline-rich protein (MPRP), which is the structural and functional counterpart of the major anionic proline-rich proteins in the parotid and submandibular secretions of man (Oppenheim, F. G., Offner, G. D., and Troxler, R. F. (1982) *J. Biol. Chem.* 257, 9271–9282). Radiosequencing of the material in anti-MPRP immune precipitates showed that the *in vitro* precursor of MPRP contained an 18-residue signal peptide. The *in vitro* precursor of MPRP was processed in dog pancreas vesicles to a form with a lower apparent M, and with an NH₂-terminal amino acid sequence identical to that of native MPRP. The phenylthiohydantoin derivatives of Ala and lle were detected at residue 9 and those of Val and Met were detected at residue 16 of the signal peptide. This indicated that the *in vitro* precursor of MPRP, which migrated electrophoretically as a single band in anti-MPRP immune precipitates, contained two different *in vitro* polypeptides derived from two different mRNAs. These results are discussed in the context of the genetic polymorphism among the major anionic proline-rich proteins in the parotid and submandibular secretions of man.

The major anionic proline-rich proteins constitute up to 30% of the protein in the parotid and submandibular secretions of man (1). The PRPs¹ comprise a family of homologous proteins that displays a genetic polymorphism with three phenotypes characterized by the presence of the protein pair, PRP I and II, the protein pair PRP I and IV, or all four PRPs (2). These proteins, together with the tyrosine-rich phosphopeptide, statherin (3), inhibit spontaneous precipitation of calcium phosphate salts and crystal growth of calcium phosphate salts in *vitro* (4). Since saliva is supersaturated with calcium phosphate salts, the biological function of the major anionic PRPs appears to be to maintain saliva supersaturated with respect to calcium phosphate and to prevent excess deposition of calcium phosphate on tooth surfaces (4, 5).

Little is known about the molecular events occurring during the biosynthesis of PRPs. Meunzer et al. (6, 7) reported that chronic treatment of rats with the β-agonist, isoproterenol, resulted in hypertrophy of the parotid glands and concomitantly, expression of the genes for six basic proline-rich proteins. The six basic proline-rich proteins were the primary translation products templated by poly(A⁺) mRNA from the parotid glands of isoproterenol-treated animals, whereas these proteins were essentially undetectable in translation reactions templated by poly(A⁺) mRNA from untreated animals (8). While the isoproterenol-treated rat parotid gland is an interesting system for investigating the effects of catecholamines on gene expression, the biological function of basic proline-rich proteins in the oral cavity of rats and man is not known.

We described the cell-free translation of the mRNAs for precursors of PRPs from a human submandibular gland and identified these precursors on the basis of cross-reactivity with immune serum specific for PRPs and preferential incorporation of radiolabeled proline (9). The disadvantages of using human parotid or submandibular glands reside in the limited availability of glandular tissue and the complexity of the system due to the genetic polymorphism among the PRPs (2).

We have isolated a proline-rich glycoprotein (MPRP) which is a major component in the parotid and submandibular secretion of the subhuman primate, *Macaca fascicularis* (10). The chromatographic and electrophoretic properties and amino acid composition of MPRP are very similar to those of

¹ The abbreviations used are: PRP, proline-rich protein; MPRP, macaque proline-rich protein; Quadrol, N,N,N',N'-tetraakis(2-hydroxypropyl)ethylenediamine, HEPES, N-2-hydroxyethylpiperazine-N-'2-ethanesulfonic acid; PTH, phenylthiohydantoin.
the PRPs. MPRP and the PRPs display a 68% homology within the NH₂-terminal 66 residues (10, 11). Kousvelari et al. (12, 13) have shown immunohistochemically at the light and electron microscopic level the numerous acinar cells of both human and macaque parotid and submandibular glands that site of prolactin-rich protein biosynthesis. MPRP and the PRPs have comparable activities in the crystal growth inhibition assay (10), leaving little doubt that MPRP is the macaque counterpart of the major anionic PRPs of man. Further, M. fascicularis is known to develop a number of human diseases under experimental conditions including periodontitis and caries (14).

In the present investigation, isolation of translatable mRNA from the macaque parotid gland and characterization of the in vitro precursor of MPRP are described.

**Experimental Procedures**

**Materials**—Parotid glands were surgically removed from adult animals within 1 h of sacrifice, frozen in liquid nitrogen, and stored at −80 °C until used.

**RNA Isolation**—Frozen tissue (approximately 8 g) was broken into small fragments by mortar and pestle under liquid nitrogen and transferred to 20 ml of extraction buffer consisting of 0.02 M Tris-HCl, pH 8.0, 0.075 M NaCl, 0.025 M EDTA and 0.5% sodium dodecyl sulfate. Subsequently, 10 ml of phenol saturated with extraction buffer and 4 ml of phenol saturated with extraction buffer, the homogenate was kept on ice for 30 min, and centrifuged at 10,000 × g for 10 min. The aqueous phase was extracted twice with an equal volume of phenol: chloroform:isoamyl alcohol (25:24:1, v/v) and twice with chloroform:isoamyl alcohol (24:1, v/v). Nucleic acids in the resulting aqueous phase were recovered by ethanol precipitation overnight at −20 °C. The RNA was further purified by repeated precipitation in the presence of 3 M sodium acetate, pH 6.0, to remove DNA and low M, RNAs (15). The resulting RNA was ethanol-preserved, twice extracted with 2 ml potassium chloride in 50 mM sodium acetate, pH 6.0, and again ethanol-precipitated. The purified RNA was chromatographed on oligo(dT)-cellulose (Type II; Collaborative Research, Waltham, MA) as described by Aviv and Leder (16). The poly(A)-enriched and poly(A)-deficient fractions (respectively referred to as poly(A)⁺ and poly(A)⁻ mRNAs, respectively) were ethanol precipitated and stored at −80 °C. The poly(A)⁺ fraction was maintained on drying agarose gels (17) and shown to contain undegraded 18 and 28 S ribosomal RNAs.

In one experiment, the poly(A)⁺ fraction was chromatographed on poly(U) agarose (P-L Biochemicals). The poly(A)⁺ fraction was applied to the column in binding buffer composed of 50 mM Tris-HCl, pH 8.0, 0.5 mM MgCl₂, 0.5 mM EDTA, 0.5% sodium dodecyl sulfate, and 25% formamide (v/v). The poly(A)⁺ fraction (not retained on the column in binding buffer) was recovered by the addition of 2 volumes of ethanol and stored at −20 °C.

**Cell-free Translation**—Reticulocyte lysates were prepared from rabbits made anemic with acetylsalicylic acid and lyses were stored under liquid nitrogen. Prior to use, lyses were made mRNA dependent by limited digestion with micrococcal nuclease (Boehringer Mannheim) as described by Peham and Jackson (18). The standard 20-μl translation reaction contained: 10 μl of nuclease-treated lysate, 12 mM HEPES buffer, pH 7.8, 0.5 mM magnesium borate, 2 μg/ml of creatine phosphate, 5 μM creatine phosphate, 110 μM potassium acetate, 0.25 mM magnesium chloride, 120 μl unlabelled amino acids minus the radiolabeled amino acid used, 1-10 μg of RNA, and 1 μCi/μl of L-[35S]methionine (Amersham Corp. Catalog No. N-90). The translation reactions contained: 1 μl of reticulocyte lysate, 2 μg of mRNA 1.2 μl of dog pancreas vesicles (New England Nuclear; 1:4 dilution of vesicles as supplied), 1 μCi/μl of L-[35S]methionine, or L-[3H]valine, L-[3H]isoleucine, L-[3H]leucine, L-[3H]phenylalanine, or L-[3H]proline (Amersham Corp. Catalog No. N-90). Translation reactions were incubated for 35 min at 30 °C, made 2% with respect to sodium dodecyl sulfate, and heated to 100 °C for 2 min. After cooling on an ice bath, 4 volumes of 10% (v/v) immunoprecipitation buffer and 2 μl of anti-MPRP immune serum were added and the mixtures were kept at 4 °C overnight. Heat-killed, formalin-fixed, S. aureus was added and the solid material was recovered by centrifugation through a 1 M sucrose solution (300 μl) in 1 × buffer. The pellet was washed repeatedly with the same buffer and translation products were eluted from the solid material in sample buffer for electrophoretic analysis or in 70% formic acid for radiosequencing.

**RNA Degradation of Translation Products**—Sequence chemicals were purchased from Beckman Instruments or Pierce Chemical Co. The immunoprecipitated translation products eluted from heat-killed, formalin-fixed S. aureus in 0.6 ml of 70% formic acid containing 1 mg of sperm whale apomyoglobin (Beckman Instruments) were applied to the spinning cup with sample application program M772. Segments of labeled Edman degradation (22) was carried out on a Beckman 890 C Sequencer equipped with a cold trap using program 121078 with 0.25 M Quadrol and a combined S2 and S3 wash. The background of each radiosequence run was reduced by performing a wash step (no coupling reagent) followed by double coupling. Phenylthiocyanoacetinorleucine was added to each fraction collector tube to serve as an internal standard.

The butyl chloride in fraction collector tubes was evaporated to dryness under nitrogen. The residues were dissolved in 1.0 ml of butyl chloride and 0.5 ml was assayed for radioactivity in a liquid scintillation spectrometer. The remaining 0.5 ml of butyl chloride was evaporated to dryness and was suspended in 0.3 ml of sodium dodecyl sulfate, and subjected to electrophoresis in a 6% polyacrylamide gel containing 10% glycerol (v/v), 2% sodium dodecyl sulfate, and 5% mercaptoethanol (sample buffer), heated at 100 °C for 2 min, and examined electrophoretically.

**Gal Electrophoresis**—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed according to Laemmli (19) on 12.5% slabs in which the ratio of acrylamide to N,N'-methylenebisacrylamide was 1:8.5. Fluorography was performed by exposing the dried gels to Kodak XAR-5 film at −80 °C.

**Immunoprecipitation**—Preparation and specificity of rabbit antisera directed against MPRP and PRP I have been described previously (20). PRP I immune serum cross-reacts with native PRPs I, II, III, and IV.

Isolation of translation products from reticulocyte lysates by immunoprecipitation was performed as described by Belford et al. (21). Briefly, 1 volume of 250 ml Tris-HCl, pH 7.5, containing 750 μM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, and 10 μM unlabeled amino acid (5 × buffer) was added to 4 volumes of translation reaction. The appropriate immune serum, (2 μl/100-μl reaction volume) was added and the mixture was allowed to stand overnight at 4 °C. Subsequently, 1 volume of 30% (v/v) heat-killed, formalin-fixed Staphylococcus aureus (The Enzyme Center, Boston, MA) was added to 2 volumes of immunoprecipitation mixture, and every 2 volumes temperature control mixture was recovered by centrifugation through a 1 M sucrose solution (300 μl) in 1 × buffer. The pellet was washed repeatedly with the same buffer and translation products were eluted from the solid material in sample buffer for electrophoretic analysis or in 70% formic acid for radiosequencing.

**Processing of the in Vitro Precursor of MPRP in Dog Pancreas Vesicles**—Macaque parotid gland poly(A)⁺ mRNA was translated in an mRNA-dependent reticulocyte lysate obtained commercially (Amersham Corp. Catalog No. N-90). The translation reactions contained: 14 μl of reticulocyte lysate, 2 μg of mRNA 1.2 μl of dog pancreas vesicles (New England Nuclear; 1:4 dilution of vesicles as supplied), and 1 μCi/μl of L-[35S]methionine, L-[3H]leucine, L-[3H]phenylalanine, or L-[3H]proline (Amersham Corp. Catalog No. N-90). Translation reactions were incubated for 35 min at 30 °C, made 2% with respect to sodium dodecyl sulfate, and heated to 100 °C for 2 min. After cooling on an ice bath, 4 volumes of 10% (v/v) immunoprecipitation buffer and 2 μl of anti-MPRP immune serum were added and the mixtures were kept at 4 °C overnight. Heat-killed, formalin-fixed, S. aureus was added and the solid material was recovered by centrifugation through 1 M sucrose as described above. Translation products were eluted from the washed pellet with sample buffer for electrophoretic examination or with 70% formic acid for radiosequencing.
RESULTS

Cell-free Translation of the in Vitro Precursor of MPRP—
Macaque parotid poly(A+)* mRNA and poly(A−) mRNA pro-
moted incorporation of L-[3H]proline, L-[35S]methionine, and
L-[3H]isoleucine into acetone-insoluble polypeptides in an
mRNA-dependent reticulocyte lysate (Table I). The stimula-
tion of radiolabeled amino acid incorporation was approxi-
mately 5 times greater per μg of RNA with poly(A+) mRNA
template. Examination of the translation products showed
that the profile of radiolabeled polypeptides was essentially
the same with either poly(A+) mRNA or poly(A−) mRNA
templates (Fig. 1). Separate rechromatography of the poly(A+)
RNA on oligo(dT)-cellulose, or of the poly(A−) mRNA on
poly(U) agarose, did not alter the profile of polypeptides
templated by either fraction or decrease template activity
(counts/min incorporated/μg of RNA).

L-[3H]Proline was preferentially incorporated into 5 poly-
peptides, designated bands I–V (Fig. 1), which represent the
major proline-rich polypeptides whose mRNA were extracted
from the parotid gland and translated in vitro. We show below
that the material in band V is the in vitro precursor of native
MPRP. MPRP is the single, major proline-rich protein in
macaque parotid secretion functionally equivalent to the hu-
man PRPs (10). We have been unable to establish which of
the native proline-rich proteins in macaque parotid secretion
correspond to the in vitro polypeptides contained in bands I–
IV. However, it is certain that these polypeptides (bands I–
IV) are not the in vitro forms of macaque parotid gland
proteins analogous to PRPs I–IV in human parotid secretion
because MPRP is the only functionally equivalent proline-
rich protein in the macaque (10).

The apparent \( M_r \) values for proline-rich proteins are erro-
neously high in gel filtration and electrophoretic systems
calibrated with globular protein standards. Somewhat more
accurate \( M_r \) values are obtained when such systems are cali-
brated with proline-rich polypeptide standards (10, 26). The
apparent \( M_r \) values of MPRP, PRPs, and the polypeptides in
bands I–V, on slab gels calibrated with both globular protein
standards and chick skin collagen \( \alpha_1 \) (I) chain CNBr peptides,
are given in Table II. These data show that the apparent \( M_r \)
values of these proteins are about 1.5 times greater when
estimated by reference to the calibration curve for globular
protein standards versus collagen CNBr peptides. The appar-
ent \( M_r \) values of native MPRP, native PRPs I or II, and
native PRPs III or IV were 19,800, 14,300, and 12,000, re-
spectively, when estimated with the collagen CNBr peptide
calibration curve. These values are in good agreement with
the \( M_r \) values calculated from the amino acid composition or
amino acid sequences of these proteins (10, 27–29). For this
reason, the \( M_r \) values of translation products subsequently
referred to are those obtained from gels calibrated with col-
lagen CNBr peptides.

Immune precipitates prepared with anti-MPRP immune
serum from the poly(A+) mRNA directed translation reactions
(Fig. 1, lanes 1–3) contained one major component with an

### TABLE I

| Isotope | RNA Fraction | Amount | Incorporation ratio: \( \mu g \) | cpm/μg RNA |
|---------|--------------|--------|---------------------------|------------|
| L-[3H]Proline | Poly(A+) | 1.6 | 261,000 | 5.8 |
| L-[3H]Proline | Poly(A−) | 7.0 | 45,000 |
| L-[35S]Methionine | Poly(A+) | 1.6 | 43,000 | 5.4 |
| L-[35S]Methionine | Poly(A−) | 7.0 | 8,000 |
| L-[3H]Isoleucine | Poly(A+) | 1.6 | 18,000 | 4.5 |
| L-[3H]Isoleucine | Poly(A−) | 7.0 | 4,000 |

*Values represent counts/min/μg of RNA incorporated into hot trichloroacetic acid-insoluble material in a 25-μl translation reaction.

![Fig. 1](http://www.jbc.org/ Downloaded from http://www.jbc.org/)

Fig. 1. Analysis of translation products from macaque parotid gland poly(A+) mRNA and poly(A−) mRNA directed translation reactions by gel electrophoresis and fluorography. Lanes 1, 2, and 3 show acetone-insoluble polypeptides from a poly(A+) mRNA directed translation reaction containing L-[3H]proline, L-[35S]methionine, or L-[3H]isoleucine, respectively. Lanes 4, 5, and 6 show acetone-insoluble polypeptides from poly(A−) mRNA directed translation reactions containing radiolabeled proline, methionine, and isoleucine, respectively. 35,000 cpm was added to each lane. Globular protein standards: bovine serum albumin, (68,000); ovalbumin (43,000); alcohol dehydrogenase (41,000); carbolic anhydrase (29,000); RNase A (13,700). Collagen cyanogen bromide peptides from chick skin collagen \( \alpha_1 \) (I) chain: CB8 (24,800); CB3 (13,800); CB6A (8,570); CB6B2 (7,910). Major proline-rich translation products are designated bands I–V.
The carbohydrate moiety is 4,150 (10, 11). The affinity of the polypeptides in bands I-IV, but these components were quantitatively insignificant in the immune precipitates. The polypeptide in band V was the least abundant proline-rich translation product (Fig. 1, lanes 1 and 4) but the main component in the anti-MPRP immune precipitate, and on this basis was designated the in vitro precursor of MPRP. Anti-MPRP immune precipitates from translation reactions templated by poly(A⁺) mRNA containing radiolabeled proline, methionine, or isoleucine were identical to those in Fig. 2 (data not given).

**Automated Edman Degradation of the in Vitro Precursor of MPRP**—Radiolabeled methionine was incorporated into the in vitro precursor of MPRP, but native MPRP does not contain methionine (30). This suggested that a methionine-containing signal peptide occurred at the NH₂ terminus of the in vitro precursor of MPRP.

Macaque parotid gland poly(A⁺) mRNA was translated in reticulocyte lysates containing L-[³⁵S]methionine, L-[³H]valine, L-[³H]isoleucine, L-[³H]alanine, or L-[³H]leucine and the material in anti-MPRP immune precipitates from these translation reactions was subjected to automated Edman degradation.

Radiosequencing of the material in the anti-MPRP immune precipitate from the translation reaction containing radiolabeled valine revealed that the PTH derivative of methionine occurred at steps 1, 13, and 16 (Fig. 3A). This is consistent with the premise that methionine occurred at residues 1, 13, and 16 of a signal peptide at the NH₂ terminus of the in vitro precursor of MPRP.

Radiosequencing of the in vitro precursor of MPRP in the immune precipitate from the translation reaction containing radiolabeled valine that the PTH derivative of valine was present at steps 8, 16, 23, and 28 (Fig. 3B). Valine is located at residues 5 and 10 of native MPRP (10, 11). Therefore, it was concluded that the PTH derivative of valine at steps 23 and 28 of the in vitro precursor of MPRP corresponded to valine at residues 5 and 10 of the native protein. If correct, this would mean that the in vitro form of MPRP contains an 18-residue signal peptide.

Radiosequencing of isoleucine- and alanine-labeled material in immune precipitates demonstrated that the PTH derivative of isoleucine was located at steps 4, 9, and 17, and that the PTH derivative of alanine occurred at steps 9 and 12 (Fig. 3 C and D). The radioactivity observed at step 1 in the analysis of isoleucine-labeled material was an artifact because no radiolabeled PTH-isoleucine was detected when the sample was examined by high pressure liquid chromatography. The PTH derivative of leucine was detected at steps 2, 3, 5, 6, 10, and 11 by radiosequence analysis of immune precipitate from a translation reaction carried out with radiolabeled leucine (Fig. 3E). From the foregoing results, a partial amino acid sequence of the signal peptide at the NH₂ terminus of the in vitro precursor of MPRP was deduced (Scheme 1).

Both methionine and valine occurred at residue 16 and both alanine and isoleucine occurred at residue 9 of the MPRP signal peptide. This can be explained if two different MPRP precursors, derived from two different mRNAs, were isolated from translation reactions with anti-MPRP immune serum. The observed microheterogeneity precluded unambiguous determination of the complete amino acid sequences of either signal peptide by radiosequencing techniques, and further experiments to detect further microheterogeneity were not performed.

**Processing of the in Vitro Precursor of MPRP**—The supposition that the in vitro precursor of MPRP contains an 18-residue signal peptide is based on assignment of PTH-[³H]valine at steps 23 and 28 to residues 5 and 10 of the native protein. This was confirmed by showing that dog pancreas vesicles processed the in vitro MPRP precursor to a polypeptide with a smaller Mr, which lacked methionine and had an apparent Mr of 11,500 (Fig. 2). This component displayed an electrophoretic mobility identical to that of the polypeptide in band V. The anti-MPRP immune serum showed a weak affinity for the polypeptides in bands I-IV, but these components were quantitatively insignificant in the immune precipitates. The polypeptide in band V was the least abundant proline-rich translation product (Fig. 1, lanes 1 and 4) but the

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**Table II**

| Sample  | Apparent Mr (kDa) |
|---------|------------------|
| MPRP    | 1.5, 16          |
| PRP I or II | 14, 26         |
| PRP III or IV | 19, 30       |
| Band I  | 22, 30          |
| Band II | 26, 30          |
| Band III | 23, 30         |
| Band IV | 20, 30          |
| Band V  | 19, 25          |

| Sample  | Apparent Mr (kDa) |
|---------|------------------|
| MPRP    | 20,000 ± 500    |
| PRP I or II | 300 ± 500      |
| PRP III or IV | 19,500 ± 400  |
| Band I  | 23,000 ± 500    |
| Band II | 26,200 ± 500    |
| Band III | 33,000 ± 500   |
| Band IV | 22,000 ± 500    |
| Band V  | 19,700 ± 150    |

*Native MPRP is a phosphoglycoprotein in which the minimum Mr of the polypeptide moiety is 15,240 and the Mr of the carbohydrate moiety is 4,150 (10, 11). The Mr values of PRP I and III calculated from the amino acid sequence are 15,627 and 11,145, respectively (27, 28). The Mr values of globular protein standards and collagen CNBr standards are given in the legend to Fig. 1.*

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**Fig. 2. Analysis of anti-MPRP immune precipitates from poly(A⁺) mRNA directed translation reactions by gel electrophoresis and fluorography.** Lane 1, acetone-insoluble polypeptides from a translation reaction containing L-[³H]proline (35,000 cpm); lane 2, anti-MPRP immune precipitate from the translation reaction shown in lane 1 (8000 cpm); lane 3, anti-MPRP immune precipitate from a translation reaction containing L-[³S]methionine (8000 cpm); lane 4, anti-MPRP immune precipitate from a translation reaction containing L-[³H]isoleucine (8000 cpm).
NH₂-terminal sequence corresponding to that of native MPRP.

Macaque parotid gland poly(A⁺) mRNA was translated in reticulocyte lysates with radiolabeled proline, methionine, or valine, with or without dog pancreas vesicles.

In the translation reaction containing radiolabeled proline and dog pancreas vesicles, processing of all proline-rich polypeptides (bands I–V) was noted (Fig. 4, lanes 1 and 2), and the apparent Mᵦ values of these polypeptides decreased from 200 to 1100 daltons when compared to proline-rich polypeptides from control translations without dog pancreas vesicles (Table III). Processing of the in vitro precursor of MPRP (band V) resulted in a decrease in the apparent Mᵦ from 11,500 to 10,500 (Fig. 4, lanes 3 and 4). In this experiment, processing of the MPRP precursor was about 80% complete as indicated by the presence of the Mᵦ = 10,500 and 11,500 bands in a ratio of approximately 4:1 (Fig. 4, lane 4). The extent to which the in vitro form of MPRP was processed by dog pancreas vesicles in the experiments depicted in Figs. 4 and 5 was 80 and 50%, respectively. The explanation for this result is not clear.

With the commercial reticulocyte lysate (Amersham Corp.) the profile of proline-rich proteins was the same as that seen using the lysate prepared according to the procedure of Pelham and Jackson (18). However, the in vitro precursor of MPRP was a much more prominent band in the commercial lysate even though the poly(A⁺) template was the same used with the other lysate (compare Fig. 1, lane 1 and Fig. 4, lanes 1 and 2). We cannot explain this observation.

Macaque parotid gland poly(A⁺) mRNA was translated in reticulocyte lysates containing radiolabeled methionine, with or without dog pancreas vesicles. In the presence of dog pancreas vesicles, the polypeptides in bands I–V were difficult to detect whereas these polypeptides were present in translation reactions minus dog pancreas vesicles (data not given) and identical to those in Fig. 1 (lane 2). Furthermore, band V was not detected in the anti-MPRP immune precipitate from the translation reaction containing dog pancreas vesicles (data not given). These results provide indirect evidence that each proline-rich polypeptide (bands I–V) contains a signal peptide with one or more methionine residues and that the processed polypeptides lack this amino acid.

Macaque parotid gland poly(A⁺) mRNA was next translated in a reaction containing L-[³H]valine and dog pancreas vesicles. Electrophoretic analysis of the anti-MPRP immune precipitate demonstrated that the in vitro precursor of MPRP had been partially processed due to the presence of both Mᵦ = 10,500 and 11,500 bands in the immune precipitate. Automated Edman degradation of the material in the immune precipitate showed that the PTH derivative of valine occurred at steps 5, 8, 10, and 16 (Fig. 5). The interpretations of these data were that the PTH-³[H]valine at steps 5 and 10 corresponded to residues 5 and 10 of the in vitro MPRP precursor from which the signal peptide had been cleaved and that the PTH-³[H]valine at steps 8 and 16 corresponded to valine at residues 8 and 16 of the signal peptide. Comparative Immunology—Macaque parotid gland poly(A⁺) mRNA and poly(A⁺) mRNA were translated in a reticulocyte lysate with L-[³H]proline as the radiolabeled amino acid. Immune precipitates were prepared using antisera directed against either MPRP or against the human proline-rich protein, PRP I. As noted above, anti-MPRP immune precipitates contained primarily the in vitro precursor of MPRP (band V: apparent Mᵦ = 11,500) (Fig. 6, lanes 2 and 4). However, anti-PRP I immune precipitates did not contain the in vitro precursor of MPRP but instead contained the proline-rich polypeptides corresponding to bands II, III, and IV with a small amount of band I polypeptide (Fig. 6, lanes 1 and 3). The reason for failure of anti-PRP I immune serum to cross-react with the in vitro precursor of MPRP, even though this anti-serum cross-reacts with native MPRP, is difficult to explain. Nevertheless, the strong cross-reactivity between anti-PRP I immune serum and the macaque proline-rich polypeptides in Bands II, III, and IV is interesting and must reflect common antigenic determinants in PRP I and the macaque proline-rich polypeptides.

**DISCUSSION**

The present investigation is the first to demonstrate cell-free translation of the mRNAs for proline-rich proteins from the parotid gland of the subhuman primate, *M. fascicularis*. One of the proline-rich translation products (band V) was shown to be the in vitro precursor of MPRP on the basis of its apparent Mᵦ, cross-reactivity with anti-MPRP immune serum, automated Edman degradation of the material in anti-
**SCHEME 1.** Alignment of the partial amino acid sequence of the in vitro form of MPRP with the NH₂-terminal amino acid sequence of the native protein. Pse, phosphoserine.

| Step | Residue: | 1 | 5 | 10 |
|------|---------|---|---|----|
| step 1 | 1 | 15 | 20 | 25 |
| step 2 | 5 | 10 | 15 | 20 |
| step 3 | 10 | 15 | 20 | 25 |
| step 4 | 15 | 20 | 25 | 30 |
| step 5 | 20 | 25 | 30 | 35 |

**MATERIALS AND METHODS**

MPRP immune precipitates, and processing in dog pancreas vesicles to a polypeptide with an NH₂-terminal amino acid precursor of MPRP contains a signal peptide at the NH₂-terminus as has been reported for numerous other secretory proteins. The proteins in vitro precursor of MPRP contains a signal peptide at the NH₂-terminus as has been reported for numerous other secretory proteins. The proteins in vitro precursor of MPRP contains a signal peptide at the NH₂-terminus as has been reported for numerous other secretory proteins.

The present work may be among the first in which mRNAs for secretory proteins have been found in both the poly(A⁺) mRNA and poly(A⁻) mRNA fractions. Parenthetically, the genes for PRPs in man are located on chromosome 1 (34). Thus, it would be expected that the genes for macaque parotid gland proline-rich proteins are also encoded in nuclear DNA and that the mRNAs derived from these genes would at some point become polyadenylated.

Second, we identified 5 major proline-rich polypeptides (bands I–V) among the translation products templated by mRNAs from the macaque parotid gland, and the polypeptide(s) in band V was unmistakably characterized as the native form of MPRP with the NHI-terminal amino acid sequence of the native protein. Pse, phosphoserine.

**TABLE III**

| Band | Apparent Mr (CNBr peptides) |
|------|--------------------------|
| I    | 25K                      |
| II   | 14K                      |
| III  | 8.5K                     |
| IV   | 8K                       |
| V    | 5K                       |

**Fig. 4.** Cell-free translation of macaque parotid gland poly(A⁺) mRNA in a reticulocyte lysate in the presence and absence of dog pancreas vesicles and with L-[³H]proline as the radiolabeled amino acid. The fluorogram shows acetone-insoluble polypeptides from translation reactions lacking (lane 1) and containing (lane 2) dog pancreas vesicles. Lanes 3 and 4 show the anti-MPRP immune precipitates from the translation reactions depicted in lanes 1 and 2, respectively. Radioactivity applied was as follows: lane 1, 275,000 cpm; lane 2, 290,000 cpm; lane 3, 19,000 cpm; lane 4, 18,000 cpm.
precipitates from a poly(A+) mRNA directed translation reaction containing dog pancreas vesicles and L-[^3H]valine. The amino acid sequences of the processed and unprocessed polypeptides are shown in the upper panel. Each Sequencer cycle was assayed as described under "Experimental Procedures" and the results are given in the lower panel. The PTH derivative of valine detected at steps 5 and 10 corresponds to valine at residues 5 and 10 of processed MPRP polypeptide; the PTH derivative of valine at steps 8 and 16 corresponds to valine at residues 8 and 16 of the signal peptide in the unprocessed precursor of MPRP. Approximately 20,000 cpm were applied to the Sequencer cup. The repetitive yield of the sperm whale apomyoglobin standard was 90%.

FIG. 5. Radiosequence analysis of the material in anti-MPRP immune precipitates from a poly(A+) mRNA directed translation reaction containing dog pancreas vesicles and L-[^3H]valine. The amino acid sequences of the processed and unprocessed polypeptides are shown in the upper panel. Each Sequencer cycle was assayed as described under "Experimental Procedures" and the results are given in the lower panel. The PTH derivative of valine detected at steps 5 and 10 corresponds to valine at residues 5 and 10 of processed MPRP polypeptide; the PTH derivative of valine at steps 8 and 16 corresponds to valine at residues 8 and 16 of the signal peptide in the unprocessed precursor of MPRP. Approximately 20,000 cpm were applied to the Sequencer cup. The repetitive yield of the sperm whale apomyoglobin standard was 90%.

First 2 bases of the codons for the latter two amino acids. We have determined approximately 75% of the primary structure of native MPRP and have found no evidence for microheterogeneity in the amino acid sequence (10, 11). This suggests the possibility that the base sequence in the mRNAs for the two in vitro precursors of MPRP may be identical in the regions coding for the native protein whereas the base sequences coding for the signal peptides are clearly different. A somewhat similar situation has been described for mouse liver and salivary gland α-amylase mRNAs which have identical base sequences in the coding and nontranslated 3′ region but different base sequences in the 5′ untranslated regions (37, 38). Microheterogeneity in the amino acid sequences of the signal peptides of other secretory proteins such as rat preproinsulin (39) and canine pretrypsinogen (40) has been described.

Finally, electrophoretic analysis of the material in anti-MPRP immune precipitates from reticulocyte lysates (Fig. 2) and automated Edman degradation of native MPRP (10, 11) indicate the presence of a single component both in the translation reactions and in the protein found in the parotid secretion of M. fascicularis, respectively. It is interesting, therefore, that radiosequencing experiments (Fig. 3) showed that the in vitro precursor of MPRP comprises two polypeptides. This suggests the possibility that there may be two genes for MPRP in M. fascicularis, as might be predicted considering the genetic polymorphism among human PRFs.

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