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CHARACTERIZATION OF PRIMARY TRANSLATION PRODUCTS FROM OVINE AND RAT SALIVARY GLAND mRNAs

R. Nichols, Don M. Carlson*, and Jack E. Dixon

Biochemistry Department, Purdue University, West Lafayette, IN 47907

*Biochemistry and Biophysics Department, University of California, Davis, CA 95616

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Ovine and rat salivary gland mRNAs have been prepared and their translation products characterized. A 60 kD translation product from ovine submaxillary and sublingual gland mRNAs is identical in mass to the ovine apomucin. Two additional ovine translation products, 25 and 40 kD, are specific to mucin-producing salivary glands. Four rat mRNA translation products are encoded by mucin-producing salivary glands (38, 44, 67, 69 kD). These polypeptides were not detected in the parotid gland mRNAs, a serous gland. Each of these products has a high level of [^{3}H]serine incorporation, a characteristic of mucins. The nature of these products suggests that they are mucins or mucin-like and that their molecular weights should approximate that of the corresponding apomucins.

The principal salivary glands are the parotid, submaxillary, and sublingual glands. Mucin is the predominant glycoprotein in the mucous secretions of these glands. Saliva contains two different types of secretion: a serous or watery secretion and a mucous secretion. The parotid glands produce entirely the serous type, the submaxillary glands produce both the serous and mucous types and the sublingual glands produce only mucous-type secretion. The mucous glycoproteins are the principal components responsible for the spectacular physical properties of mucous secretions. The function of mucin, along with similar glycoproteins found in the respiratory, gastrointestinal and reproductive tracts is to both lubricate and protect epithelial cells from contact with the external environment.

The oligosaccharide units of mucous glycoproteins have been studied extensively (1,2). However, there is little detailed biochemical data and very little, if any, molecular biological information available concerning the polypeptide chain of mucous glycoproteins or apomucins. The three salivary glands provide the advantage of having similar, yet distinctive compositions of secretions. These secretory proteins are reflective of the respective mRNAs present in these three glands both for the mucin polypeptide chain and the glycosyltransferases associated with glycosylation of the apomucin.

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Ovine salivary glands were chosen for study because partial amino acid sequence data for ovine submaxillary apomucin is the only known published primary structural data available for a mucous glycoprotein. The molecular weight and amino acid composition of the purified ovine submaxillary apomucin have been determined. Ovine submaxillary apomucin has a molecular weight of 58,300 (3). The amino acid compositions of ovine and rat mucins are similar to compositions reported for other mucins. The serine and threonine content of mucous glycoproteins are considerably higher than in most other proteins (3). In addition, reported amino acid compositions indicate that the rat salivary mucin is considerably enriched in alanine and glycine (4).

The results of this study represent a preliminary identification of potential mucin and mucin-like mRNAs as their corresponding cell-free translation products from ovine and rat salivary glands.

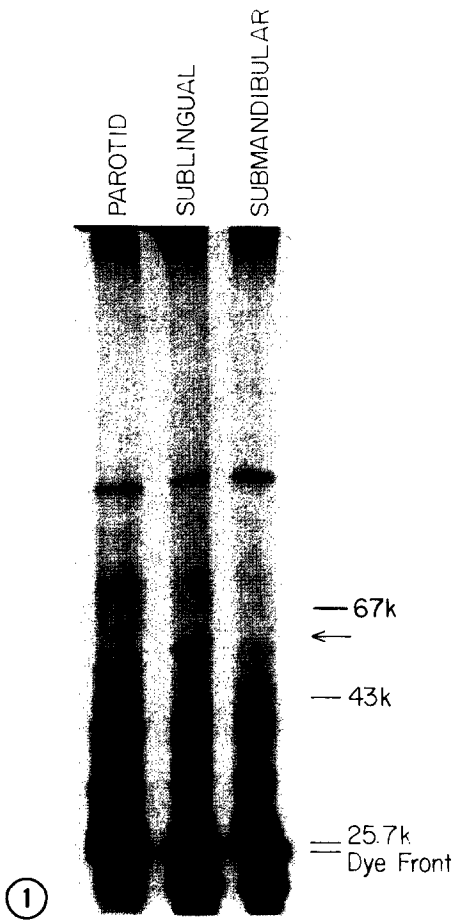
MATERIALS AND METHODS

Salivary gland tissue was removed immediately upon death and quickly frozen. A modification of the guanidine isothiocyanate method of mRNA preparation (5) was used to prepare total mRNA from the parotid, submaxillary and sublingual glands. The volume of homogenization buffer to tissue weight was 30 mls to 0.3 g, the size of the CsCl cushion was 1.5 mls, and a phenol/chloroform extraction of the redissolved RNA pellet was done.

Cell-free translations of total mRNAs were performed with the wheat germ protein-synthesizing system (6) in the presence of [^{35}S]methionine (NEN, NEG.009T), [^{3}H]serine (Amersham, TRK.308), [^{3}H]alanine (ICN, # 20005), or [^{3}H]glycine (Amersham, TRK.71). Translation products were analyzed by 10% SDS-polyacrylamide slab gels (7). In order to compare the translation patterns of the various mRNAs, an equal amount of incorporated label was applied to each sample lane of a single gel. Aliquots of each translation were measured for incorporation of radiolabel by scintillation counting (Beckman, Model LS 8100). To calibrate the gel, prestained molecular weight standards (BRL, 6040SA) were electrophoresed under the same conditions as the translation products. The gels were processed with EN^{3}HANCE (NEN) for fluorographic detection of the translation products.

RESULTS AND DISCUSSION

Mucins are highly glycosylated, often containing 60-80% carbohydrate, which is largely responsible for their physical and chemical properties. Studies of the apomucins have been hampered by the inability to remove all carbohydrate moieties from the intact polypeptide backbones. In order to learn more about the apomucins this initial study has been directed towards the characterization of cell-free translation products from ovine and rat salivary gland mRNAs. It was necessary to modify the guanidine isothiocyanate method of mRNA preparation (5) in order to prepare mRNA from the ovine and rat mucin-producing salivary glands. Preparation of mRNA from the mucin-producing salivary glands using the guanidine isothiocyanate method resulted in mRNA unsuitable for cell-free translation. Analysis of the mRNA preparations by gel electrophoresis indicated



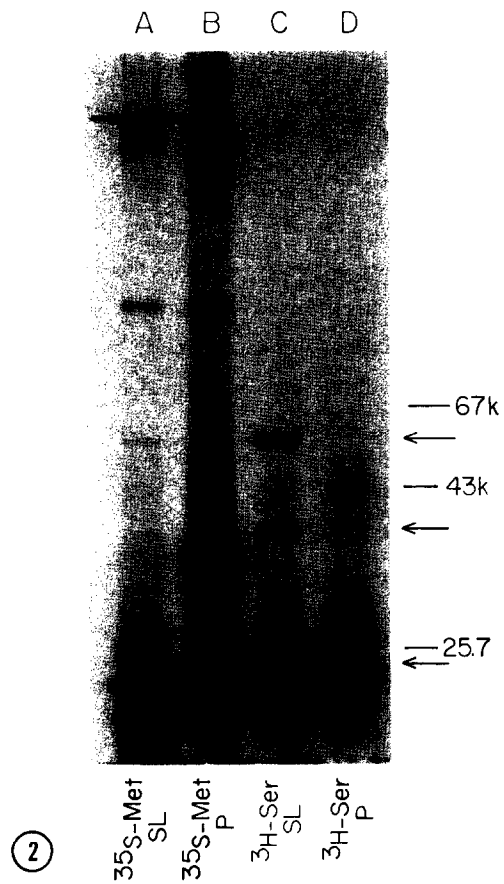


Figure 1. Electrophoretic analysis of translations from ovine salivary glands in the presence of [^{35}S]methionine. Arrow indicates a 60 kD polypeptide.

Figure 2. Electrophoretic analysis of translations from ovine parotid (P) and sublingual (SL) glands. Lane A, translation of SL mRNA using [^{35}S] methionine (^{35}S Met). Lane B, translation of P mRNA using ^{35}S Met. Lane C, translation of SL mRNA using [^{3}H] serine (^{3}H Ser). Lane D, translation of P mRNA using ^{3}H Ser. Arrows indicate serine-rich polypeptides.

that the mRNA was intact. However, cell-free translation of the mRNA resulted in incorporation of radiolabel no higher than background levels. Control experiments indicated that the poor incorporation was not due to the cell-free translation system. Modifications of the isothiocyanate procedure resulted in the isolation of intact mRNA from the submaxillary and sublingual glands suitable for translation. The volume of homogenization buffer to tissue weight was increased, due to the very viscous nature of the extract from the mucous secreting salivary glands. The size of the CsCl cushion was increased to prevent contaminants from pelleting with the mRNA. A phenol/chloroform extraction of the redissolved mRNA pellet was introduced into the procedure.

These modifications afford a reproducible method of obtaining mRNA suitable for cloning as well as for analysis by cell-free translation.

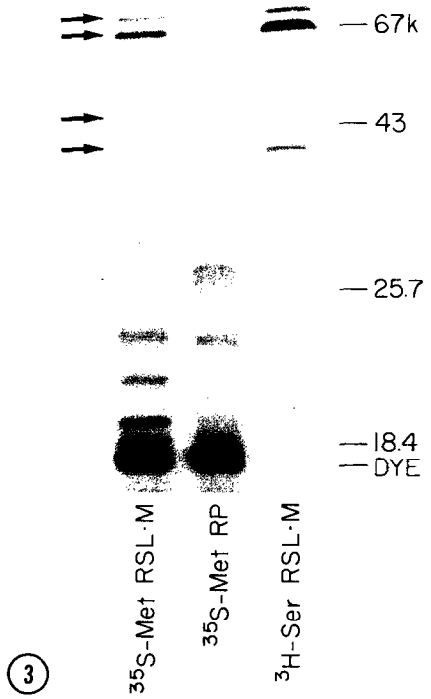
Cell-free translations of mRNA have been performed with the wheat germ protein-synthesizing system in the presence of [^{35}S]methionine and the translation products have been analyzed by SDS-polyacrylamide gel electrophoresis. The translation products from ovine salivary glands labeled with [^{35}S]methionine are shown in Figure 1. A 60,000 dalton translation product was observed for mRNAs from the submaxillary and sublingual glands, both of which are mucin-producing salivary glands. This molecular weight is comparable to the 58,300 reported for apomucin of ovine submaxillary glands (3). This polypeptide was not detected in the parotid gland mRNA translation pattern (Figure 1).

Proteins from cell-free translations which have unusual amino acid compositions can be identified on SDS-polyacrylamide gels by selective incorporation of radiolabeled amino acids. For example, the serine and threonine contents of mucous glycoproteins are considerably higher than in most other proteins (3). The serine content of ovine apomucin is 18% (3). Therefore, introduction of [^{3}H]serine into the cell-free translation mixture should result in a disproportionate incorporation of label into apomucin.

Figure 2 shows the translation products of parotid and sublingual gland mRNAs using [^{35}S]methionine and [^{3}H]serine. The [^{3}H]serine incorporation is quite striking. The 60,000 dalton [^{35}S]methionine-labeled translation product present in mucin producing salivary glands shows a high level of [^{3}H]serine incorporation relative to other products (Figure 2). This polypeptide is absent in translations of parotid mRNA (Figure 2).

Two additional polypeptides are encoded specifically by sublingual mRNAs. The [^{35}S]methionine-labeled translation products include polypeptides of 40,000 and 25,000 daltons (Figure 2). The incorporation of [^{3}H]serine into these polypeptides is characteristic of mucins. These products are absent in the translations of parotid mRNA using [^{35}S]methionine or [^{3}H]serine (Figure 2).

Is this a general pattern that can be used to identify mucin or mucin-like proteins in other species? Can the serine:methionine incorporation observed for the ovine translation products also be observed in other species, and can this information be used to provide preliminary data concerning putative mucins or mucin-like polypeptides? Translations of mRNA from the rat parotid and submaxillary and sublingual glands were done. Four translation products of molecular weights 69,000, 67,000, 44,000, and 38,000 appear to be encoded specifically by the mucin-producing salivary glands (Figure 3). These products are absent in the translations of parotid mRNA using either [^{35}S]methionine or [^{3}H]serine. Data indicate that the rat salivary mucins are considerably



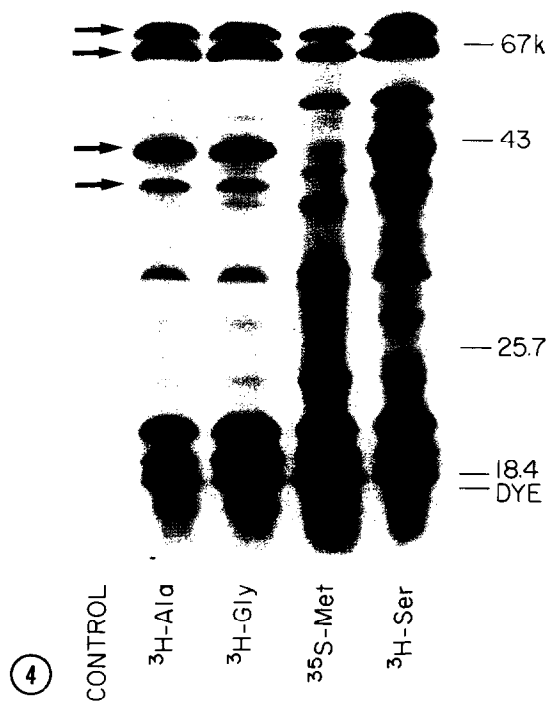


Figure 3. Electrophoretic analysis of translations from rat parotid (RP) and sublingual-submaxillary glands (RSL.M) using [^{35}S]methionine, (^{35}S-Met) and [^{3}H]serine, (^{3}H-Ser). Arrows indicate serine-rich polypeptides.

Figure 4. Electrophoretic analysis of translations from rat sublingual-submaxillary glands using [^{3}H]alanine, (^{3}H-Ala); [^{3}H]glycine, (^{3}H-Gly); [^{35}S]methionine, (^{35}S-Met); and [^{3}H]serine, (^{3}H-Ser). Arrows indicate serine-rich polypeptides.

enriched in alanine and glycine (4). These four translation products also show a high level of incorporation of [^{3}H]alanine and [^{3}H]glycine (Figure 4).

The high level of serine incorporation and apparent specificity to mucin-producing salivary glands suggest that these translation products represent mucins or mucin-like polypeptides. The molecular weights of the translation products should provide approximate values for the corresponding apomucins.

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